Swedish NMR meeting 2022, October 4-6

Wallenberg Conference Center, Medicinaregatan 20A, Göteborg

www.gu.se/nmr/english/events/swedish-nmr-meeting-2022

	------- **Tuesday October 4** ------
11.00 – 12:00	Registration, poster mounting
12.00 –13.00	*Lunch served at Lyktan*
13.00 –13.10	***Opening & Welcome*** *(Göran Karlsson, Director of SwedNMR)*
13.15 – 14.00	***New Developments in Small Molecule NMR: From Fast-Pulsing 2D Experiments to Flexibility Determination Using Partially Aligned Samples*** *Burkhard Luy, Karslruhe Institute of Technology*
Session 1	*"Small molecules", Chair: Mattias Hedenström*
14.00 – 14.30	***Expanding the Boundaries of Characterising Weak Interactions in Dilute Solutions*** *Mate Erdelyi, Uppsala University*
14.30 – 15.00	*Coffee*
15.00 – 16.00	Selected presentations, 3x20 minutes
	Substrate binding and protonation states at the active-site of a nickel superoxide dismutase-derived metallopeptide: implications for the mechanism of superoxide degradation *Daniel Tietze, University of Gothenburg* ***In-Cell Quantification of Drugs by MAS DNP NMR*** *Staffan Schantz, AstraZeneca and the University of Gothenburg* ***Characterization of a new rotenoid from the leaves of Millettia oblata ssp. teitensis*** *Ivan Kiganda, University of Nairobi and Uppsala University*
16.00– 16.30	***Isotopically labelled carbohydrates for NMR studies of protein-carbohydrate interactions*** *Gustav Nestor, Swedish University of Agricultural Sciences*
16.30 – 18.00	Bruker user meeting (I) ***Welcome*** *(Dick Sandström)* ***Magnet and Probe Updates*** *(Andrew Gibbs)* ***Developments in Metabolomics NMR*** *(Claire Canet)* ***News and Updates on Recent Bio-NMR Experiments and TopSpin Features*** *(Maksim Mayzel)*
18.00 - 20.30	*Informal mixer & Poster session*
	------- **Wednesday October 5** ------
Session 2	*"BioNMR (I) ", Chair: Vladislav Orekhov*
08.30 – 09.15	***Expanding the molecular basis for sugar recognition in the malarial sugar transporter*** *David Drew, Stockholm University*
09.15 – 10.15	Selected presentations, 3x20 minutes
	Structural basis of substrate recognition and allosteric activation of the pro-apoptotic mitochondrial HtrA2 protease *Emelie Aspholm, University of Gothenburg* Unravelling Bcl-2 proteins´ functioning at mitochondrial membrane level *Gerhard Gröbner, Umeå University* The MYC oncoprotein directly interacts with its chromatin cofactor PNUTS to recruit PP1 phosphatase *Alexandra Ahlner, Linköping University*
10.15 – 10.45	*Coffee*
10.45 – 11.15	***The tale of loop-mutations: How a natural variant of the SARS-CoV-2 nucleocapsid protein enables detailed studies of the full-length protein by solution NMR spectroscopy*** *Irena Matečko-Burmann, University of Gothenburg*
11.15 – 12.15	Selected presentations 3 x 20 minutes
	Novel NMR Assignment strategy for large proteins with IDPs *Peter Agback, Swedish University of Agricultural Sciences*

	In-situ characterization of the bacterial outer membrane protein A *Johannes Thoma, University of Gothenburg* ***HR MAS NMR-based metabolomics – prostate cancer as case*** *Ilona Dudka, Umeå University*
12.30 – 13.30	*Lunch served at Lyktan*
Session 3	***"BioNMR (II) ", Chair: Göran Karlsson***
13.30 – 14.00	***Through hardship to the multidimensional NMR and back again*** *Vladislav Orekhov, University of Gothenburg*
14.00 – 15.00	Selected presentations 3 x 20 minutes
	SUN: Band-Selective Suppression of Unwanted Signals in Complex Mixtures *Elin Alexandersson, Swedish University of Agricultural Sciences* ***Metabolomics as a readout of the genotype*** *Anders Malmendal, Roskilde University* ***Water transport in paper-based packaging materials and other examples of MRI and NMR in chemical engineering*** *Diana Bernin, Chalmers University of Technology*
15.00 – 16.30	*Coffee, poster session and/or visit at the Swedish NMR Centre*
16.30 – 17.00	***Automated metabolite quantitation by ^{1}H-NMR*** *Hanna Eriksson Röhnisch, Swedish University of Agricultural Sciences*
17.00 – 18.00	Bruker user meeting (II) ***Game Changing Sensitivity Enhancement with the CPMAS CryoProbe*** *(Alia Hassan)* ***Benchtop NMR Updates*** *(Jens Chr. Madsen)* ***Service News*** *(Nils Nyberg)*
18.30 – 21.00	*Meeting Dinner at Restaurant Lyktan*
	------- **Thursday October 6** ------
08.30 – 09.15	***How NMR spectroscopy can help close the phosphate cycle*** *Ulla Gro Nielsen, University of Southern Denmark*
Session 4	***Solids and Materials NMR, Chairs: Tobias Sparrman & Sergei Dvinskikh***
09.15 – 10.15	Selected presentations 3 x 20 minutes
	Studying modified cellulose materials using DNP-NMR spectroscopy *Hampus Karlsson, Chalmers University of Technology* ***The right answer for the right reason: towards reliable predictions of paramagnetic NMR shifts*** *Aleksander Jaworski, Stockholm University* ***Hydrogen bonding in bulk and nanoconfined ionic liquid crystals*** *Sergei Dvinskikh, Royal Institute of Technology*
10.15 – 10.45	*Coffee*
10.45 – 11.15	***The fate of Li ions in the carbon electrodes of Li-ion batteries*** *Istvan Furo, Royal Institute of Technology*
11.15 – 12.15	Selected presentations 3 x 20 minutes
	A first peek at the Nordic Algae Cell Wall Structure *Tobias Sparrman, Umeå University* ***Multi-scale structural investigations of protein materials*** *Christofer Lendel, Royal Institute of Technology* ***MAS DNP NMR for Pharmaceutical Formulation Characterization*** *Arthur C. Pinon, University of Gothenburg*
12.30 – 13.30	*Lunch served at Lyktan*
13.30 – 14.15	***Solid-State NMR of Pharmaceuticals and Plant Cell Walls*** *Steven Brown, Warwick University*
14.15 – 14.30	Closing remarks (*tba*)

Scientific committee: SwedNMR module groups & Mattias Hedenström, Sergei Dvinskikh, Vladislav Orekhov, Göran Karlsson
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Abstracts, talks and posters, alphabetical order

Novel NMR assignment strategy for IDPs and large proteins containing disordered domains

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In recent years, intrinsically disordered proteins (IDPs) and disordered domains in large proteins have attracted great attention. Many of them contain linear motifs that mediate interactions with other factors during formation of multicomponent protein complexes. NMR spectrometry is a valuable tool for characterizing this type of interactions. We propose a robust and reasonably quick protocol to assign the backbone and sidechain resonances in NMR spectra of large IDPs in free or in complex with structured domains. It assumes a twostep process: first, assignment of the unfolded protein in the presence of denaturant, for example: GdmCl. The significantly improved quality of the NMR spectra allows the use of a combination of experiments defining the type of amin acid and accelerated data acquisition using NUS H(N)-type 3D Target Acquisition (TA) experimental approach. To make MUSIC type experiments more efficient, we made modifications to the available pulse sequences with (a) semiconstant time functions to boost resolution in the 15N dimension (b) TROSY and (c) sensitivity improvement options. Second, back titration from the unfolded protein to its native form allows the transfers of amide resonances assignments. This protocol was applied to the structural study of fully disordered nsP3 HVDs of Venezuelan equine encephalitis virus (VEEV) and the SARS-CoV-2 full length nsp1 protein including the folded domain together with the flaking N- and C- terminal intrinsically disordered fragments.

Novel NMR Assignment strategy for large proteins with IDPs.

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Abstract:

In recent years, intrinsically disordered proteins (IDPs) and disordered domains in large proteins have attracted great attention. Many of them contain linear motifs that mediate interactions with other factors during formation of multicomponent protein complexes. NMR spectrometry is a valuable tool for characterizing this type of interactions on both amino acid (aa) and on atomic levels. We propose a robust and reasonably quick protocol to assign the backbone and sidechain resonances in NMR spectra of large IDPs in free or in complex with structured domains. It assumes a two step process: first, assignment of the unfolded protein in the presence of denaturant, GdmCl. The significantly improved quality of the NMR spectra allows the use of a combination of experiments defining the type of aa and accelerated data acquisition by NUS of H(N)-type 3D Target Acquisition (TA) experimental approach. To make MUSIC type experiment more efficient, we made modifications to the available pulse sequences with (a) semiconstant time functions to boost resolution in the 15N dimension (b) with TROSY and (c) sensitivity improvement options. Second, back titration from the unfolded protein to its native form allows the transfers of amide resonances assignments. This protocol was applied to the structural study of fully disordered nsP3 HVDs of Venezuelan equine encephalitis virus (VEEV) and the SARS-CoV-2 full length nsp1 protein including the folded domain together with the flaking N- and C- terminal intrinsically disordered fragments.

The MYC oncoprotein directly interacts with its chromatin cofactor PNUTS to recruit PP1 phosphatase

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cMyc is one of the most dysregulated oncogenes in all cancer forms and therefore it has for long time been one of cancer researchers “holy grails” to inhibit cMyc. To our knowledge, no-one has been able to develop a drug with this purpose, probably due to cMyc’s intrinsically disordered regions. To address this, we have previously investigated the proxiome of cMyc to find interactions with the potential to inhibit cMyc’s interactions crucial for cancer. One of the identified interactors were the protein phosphatase-1 nuclear-targeting subunit (PNUTS). (Kalkat, 2018) If this interaction is inhibited cMyc gets hyperphosphorylated and degraded. (Dingar, 2018). We have determined the first NMR structure of PNUTS 1-148(called PAD) revealing an alpha helical structure with slightly disordered N-terminal. An N-terminal part of cMyc called myc box zero binds in a highly dynamical way to the C-terminal part of PAD, shown both by titration experiments (NMR and bio-layer interferometry), a myc box zero-PAD fusion structure (NMR), mutational studies and Rosetta modelling. These data demonstrate that the MB0 region of MYC directly interacts with the PAD of PNUTS, which provides new insight into the control mechanisms of MYC as a regulator of gene transcription and a pervasive cancer driver.

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Wei Y., Redel C., Ahlner A., et. al. The MYC oncoprotein directly interacts with its chromatin cofactor PNUTS to recruit PP1 phosphatase Nucleic Acids Res. 50(6):3505-3522 (2022)

Dingar D. et al, MYC dephosphorylation by the PP1/PNUTS phosphatase complex regulates chromatin binding and protein stability, Nat Commun. 9(1):3502 (2018)

Kalkat M. et al MYC Protein Interactome Profiling Reveals Functionally Distinct Regions that Cooperate to Drive Tumorigenesis Mol Cell. 72(5):836-848 (2018)

SUN: Band-Selective Suppression of Unwanted Signals in Complex Mixtures

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Biological samples, e.g. plant extracts, blood, and urine, typically contain numerous different metabolites with large variations in concentration. When such samples are analyzed with NMR spectroscopy, the resulting spectra are often very complicated with severe spectral overlap and dynamic range problems. This may obstruct the identification and analysis of certain metabolites, in particular those of low concentration. To study low-abundant metabolites, sample pre-treatment such as chromatography is therefore often needed before the NMR analysis. However, this is undesirable as it is time-consuming, costly and disturbs the integrity of the sample.

We have developed an NMR experiment called SUN (Suppression of UNwanted signals) that can be applied to intact samples and allows signals from abundant or otherwise problematic compounds to be removed from selected regions of NMR spectra [1]. Other signals in the selected spectral region are retained, provided that they are *J*-coupled to at least one signal located outside of the targeted region. Thus, SUN is useful for identifying and characterizing low-abundant compounds in samples containing other compounds in high concentration. The approach has been successfully applied to various different samples containing large amounts of sugar, including artificial mixtures, orange juice, and plant samples.

1. Alexandersson, Elin; Sandström, Corine; Lundqvist, Lena C.E.; Nestor, Gustav, RSC Advances, 10(54), 32511–32515, 2020

Structural basis of substrate recognition and allosteric activation of the pro-apoptotic mitochondrial HtrA2 protease

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HtrA2 (High temperature requirement protein A2) is a mitochondrial serine protease of the HtrA family found in all kingdoms of life[1]. HtrA2 resides in the inner mitochondrial membrane where it exerts its role in protein quality control, and acts as a proapoptotic factor when released into the cytosol where it binds and cleaves inhibitor of apoptosis (IAP) proteins such as XIAP[2]. HtrA2 has been implicated in several neurodegenerative diseases such as Parkinson's and Alzheimer's disease as well as several different cancer types[1,3–6]. Due to its importance in protein quality control and its connection to several severe human diseases, HtrA2 is an important target of study. However, the functional cycle, interaction partners as well as the regulation of HtrA2 remain mostly elusive. We have used advanced solution NMR spectroscopy methods employing methyl-TROSY approaches together with biophysical characterization and biochemical assays to show how HtrA2 is allosterically activated *via* its PDZ domain. We show that upon binding of an activating peptide, HtrA2 undergoes extensive structural changes involving the regulatory loops as well as the N-terminal α-helix of the protease domain. Further, we reveal that divalent metal ions can both positively and negatively modulate the activity of HtrA2, leading to a refined model of HtrA2 regulation within the apoptotic pathway.

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Water transport in paper-based packaging materials and other examples of NMR and MRI in chemical engineering

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In 2030, 8 years from now, the emission of greenhouse gases needs to be significantly reduced to limit the rise in global temperature to 1.5°C and avoid the most severe consequences of climate change. Sweden decided to be climate-neutral in 2045, 5 years ahead of the EU. These goals require action and change of habits for all of us—a new mindset. Solutions to reach these goals are electrification of transport, reducing and substituting the number of fossil-based products and efficient use of resources. Many fossil-based products are made of plastic due to its outstanding properties of being non-degradable, water-resistant, processable, and cheap. However, the substitution of these materials to paper-based materials is challenging due to the inherent hydrophilic properties of cellulose. Other actions are utilizing industrial and agricultural waste streams for (i) sustainable textile fibers production and/or CO_2 capture as well as the development of energy-efficient processes for bio-based chemicals based on light. Here we summarize the ongoing and upcoming research using solid-state and high-field NMR and MRI.

Development of new antimicrobial peptides from marine *actino*bacteria

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Antimicrobial resistance is a serious and global health threat. In order to combat antimicrobial resistance new drugs with novel modes of action are urgently needed.[1] Antimicrobial peptides (AMPs) have shown great potency as a new class of antibiotics in the last years.[2, 3] This project focuses on novel AMPs using naturally sourced drug discovery. Non-ribosomal peptide sequences were predicted from *actino*bacteria isolated from marine sediments in Chilean *Comau fjord*. Genome sequencing of isolated *actino*bacteria lead to an ambiguous peptide sequence. Based on this core sequence three different peptide sequences were designed and synthesized in their linear and cyclic forms by automated Fmoc-based SPPS and manual on-resin head-to-tail cyclization. All peptides were subjected to antimicrobial testing against gram-positive bacteria (*S. aureus*), gram-negative bacteria *(E. coli*) and yeast (*C. albicans*). A distinct antimicrobial activity was observed for two linear and moderate activity was observed for one cyclic peptide. To understand their structure-activity relationships, characterization of the secondary structure and mode of action studies were performed. The secondary structures were analyzed by CD and NMR spectroscopy. Due to the high degree of non-natural amino acids in those peptides' NMR assignment and structure calculation protocols had to be thoroughly optimized using CCPNMR and Yasara softwares.

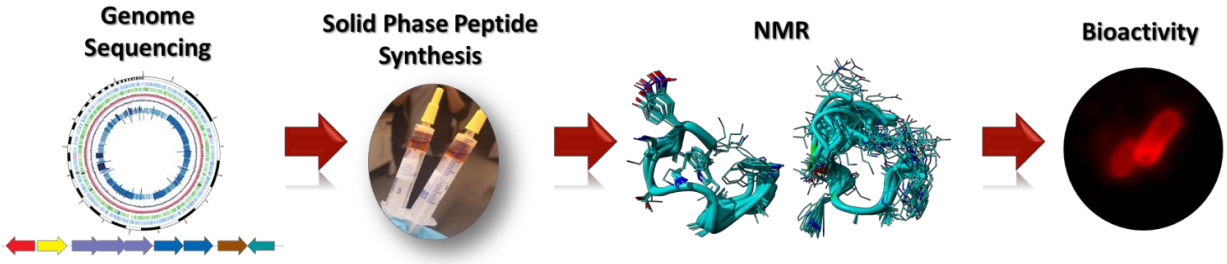

Figure 1: Flow chart: Development of new antimicrobial peptides from marine *actino*bacteria.

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Solid-State NMR of Pharmaceuticals and Plant Cell Walls

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Solid-State magic-angle spinning (MAS) NMR is a valuable tool in the characterisation and study of active pharmaceutical ingredients (APIs) [1-4]. Heteronuclear ^{1}H-^{13}C correlation experiments are invaluable for assignment, while homonuclear ^{1}H-^{1}H double-quantum (DQ) single-quantum (SQ) experiments reveal proximities (typically under 3.5 Angstroms) among pairs of hydrogen atoms. In addition, ^{14}N-^{1}H spectra show one-bond NH connectivities or additionally longer-range NH proximities depending on the recoupling time employed. In the emerging NMR crystallography concept (recognized as a sub area by the International Union for Crystallography and, in the UK, by funding for a collaborative computational program for NMR crystallography, www.ccpnc.ac.uk), experimental solid-state NMR is complemented by first-principles calculations of NMR parameters using the GIPAW (gauge-including projector augmented wave) density-functional theory planewave approach that is particularly suited to periodic solids. In addition, the talk presents the application of ^{13}C-^{13}C refocused INADEQUATE DQ-SQ to plant cell walls incorporating ^{13}C labelling [5,6].

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Expanding the molecular basis for sugar recognition in the malarial sugar transporter

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Glucose is the most important sugar for life on earth, and elucidating the coupling between sugar recognition and translocation is a fundamental question in transport biology. The hexose transporter from the malarial parasite *Plasmodium falciparum Pf*HT1, was recently captured in an occluded conformation, which has proven to be insightful state for modelling the transition-state in mammalian glucose (GLUT) transporters. In the occluded *Pf*HT1 structure, the extracellular gating helix TM7b was found to have fully closed over the sugar-binding site, indicating a strong allosteric coupling between sugar binding and gating. However, due to its weak affinity for sugars (> 1mM), it was not possible to assess sugar binding by routine methods. Here, we have probed the interaction of D-glucose and D-fructose sugars to *Pf*HT1 incorporated into liposomes by saturation-difference NMR (STD-NMR) spectroscopy. We demonstrate that residues located on TM7b, even those 15 Å from D-glucose, are just as critical for sugar-binding as residues coordinating the sugar. We have further determined the crystal structure of *Pf*HT1 in complex with 2,5-dihydromannitol, which shows how a strictly conserved asparagine residue, located in the beginning of TM7b, connects sugar binding to the gating helix in an analogous manner to D-glucose. We conclude the TM7b gate can be considered an extension of the sugar-binding pocket, which is likely required to form the high-affinity transition-state to catalyse substrate translocation.

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3. unpublished

HR MAS NMR-based metabolomics - prostate cancer as a case

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Prostate cancer (PC) is the most prevalent cancer amongst men and significant cause of morbidity and mortality in men. The disease has very heterogeneous phenotypes ranging from indolent asymptomatic cases to very aggressive life-threatening forms. We present results of our two studies, where we applied ^{1}H HR MAS NMR-based metabolomics in to the research of prostate cancer. The key advantage of this platform is that tissue remain intact after analysis, so subsequent analysis is possible. Results of our first study showed metabolite patterns significantly differed between PC and benign tissue and between samples with high and low Gleason score (GS). Five key metabolites were identified, who were sufficient to differentiate between cancer and benign tissue and between high to low GS. Additionally, we presented that two PC subclasses, TMPRSS2-ERG rearrangement-positive and -negative, are characterized by different metabolomics profiles. These results were validated with proteomic data from separated patients' cohort. In our second ongoing study we focused on molecular subtypes of PC and we aimed to verify the clinical and biological relevance of three prostate cancer subtypes. Obtained metabolomic profiles of three PC subtypes reflected molecular characterization of PC subgroups and our results suggested that the subtype-specific treatment strategies should be developed.

Hydrogen bonding in bulk and nanoconfined ionic liquid crystals

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Ionic liquid crystals (ILCs) have the typical characteristics of ionic liquids and, at the same time, a nanoscale-organized structure of liquid crystals.[1] The formation of liquid-crystalline phases in ionic liquids is driven by a balance between hydrogen bonding, electrostatic interactions, and dispersion forces. A small shift of the force balance can lead to discernible changes in phase behaviour and local molecular ordering and dynamics. Correlations have been revealed between the orientational order, mesophase window, and physicochemical properties of the anions such as ionic radius, charge delocalization, and the ability for hydrogen bonding.[1-3] Modification of the H-bonding network by hydration further contributes to mesophase stabilization in ILCs despite decreasing the orientational order and accelerated ion translational dynamics.[3,4] H-bonding interactions have also been widely studied in nanoconfined ionic liquids, presenting a class of hybrid composites combining the functional properties of ionic liquids and porous solids. Due to hydrogen bonds and other non-covalent interactions with the solid interface, confined ionic liquids exhibit distinct structural, orientational, and dynamic preferences different from the bulk.

Previously we have investigated the dynamic and the orientational ordering behaviour of ILCs using heteronuclear dipolar NMR spectroscopy and pulsed-field-gradient diffusion NMR.[2-6] In the present work, we discuss cation–anion hydrogen-bonding interactions in imidazolium-based ionic liquid crystals. We apply solid-state ^{1}H NMR to access proton chemical shift interactions in H-bonding centers.[7] Hydrogen bonding effects are compared in isotropic, liquid crystalline, and solid phases of ILCs as well as in the nanoconfined state. The influence of anion properties, cation structures, ion dynamics, and orientational order on hydrogen bond strength is discussed.

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Expanding the Boundaries of Characterising Weak Interactions in Dilute Solutions

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Noncovalent interactions are ubiquitously important for chemical and biological processes. As they are weak, their characterisation in dilute solutions is typically challenging. In this talk, I will discuss strategies that expand the current boundaries of the NMR spectroscopic detection and detailed geometric and thermodynamic characterization of weak non-covalent interactions in solution. The scope of these techniques will be demonstrated on the NMR spectroscopic description of single weak (K_a < 10 M) halogen and hydrogen bonds in dilute solutions (< 5 mM), which would not be possible with standard NMR titration techniques.

In Vitro Phosphorylation of the Intrinsically Disordered N-terminus (TAD) of c-Myc Using Difference Serine/Threonine Kinases

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Myc is a family of oncogenic, intrinsically disordered, transcription factors that have been estimated to bind up to 15% of all genes and regulate cellular activities such as cell cycle regulation, proliferation, differentiation, and cell death. Myc proteins are highly implied in different cancer types and some form of Myc deregulation has been estimated in up to 70 % of human malignancies.

The lifecycle of Myc proteins in cells is tightly regulated through phosphorylation of Threonine 58 and Serine 62. Phosphorylation of Serine 62 activates Myc which leads to sequential phosphorylation of Threonine 58, inducing ubiquitination and degradation through the proteasome. Inhibition of Threonine 58 phosphorylation through mutation has been shown to be a common cause of cancers such as Burkitt's lymphoma and AIDS-related lymphomas.

Here we present secondary structural information and cis-trans conformation of prolines as an effect of mono- or diphosphorylation of the c-Myc(1-88)S71A construct. Variously phosphorylated c-Myc variants for NMR analysis were produced using our newly developed procedures. Results obtained from intensity measurements and CheSPI structure predictions show that there is an increase in structure upon phosphorylation of c-Myc, not only around the phosphorylation sites, but several other parts seem to be indirectly influenced by the process. Analysis of the proline residues showed that all prolines were of trans conformation in the diphosphorylated form. Our results indicate that phosphorylation alone is not sufficient to induce the cis-trans conformational changes suggested to drive MYC degradation.

Towards understanding protein quality control of integral membrane proteins by the bacterial metalloprotease FtsH

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FtsH is an essential ATP-dependent protease in *Escherichia coli* (*E. coli)* responsible for degradation of unfolded or aggregated proteins as a part of the protein quality control machinery. It is an integral membrane protein anchored to the inner membrane of the bacterial cell where it forms large complexes with other membrane proteins such as QmcA and HflkC. FtsH is a metalloprotease dependent on stimulation of zinc at the active site for proteolytic activity. Substrates of FtsH include both membrane-bound proteins as well as cytosolic proteins [1]. No common substrate recognition motif could so far be established and the degron can be located at termini or at internal sites of the substrate. The most important function of FtsH is the degradation of LpxC which is responsible for regulation of lipopolysaccharide biosynthesis [2]. FtsH assembles into a homohexamer with a ring-like structure. Each monomer includes a periplasmic amino-terminal region of approximately 70 amino acids and a large carboxy-terminal cytoplasmic region, approximately 520 amino acids, which are anchored to the membrane by 2 transmembrane helices. The cytoplasmic region constitutes the ATPase domain, responsible for substrate unfolding and translocation, and the protease domain, which exhibits the proteolytic activity [3]. The active sites are localized in the center of the ring-shaped domains and substrates can only enter through a narrow pore. [4]. Although FtsH plays a vital role in *E. coli* and possibly could act as a novel antibiotic target, still, very little is known about the detailed structure, substrate recognition and its mechanism of action. To gain further insight into this membrane protease we are studying the structure and the dynamic properties of FtsH at the atomic level using solution NMR techniques.

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The fate of Li ions in the carbon electrodes of Li-ion batteries

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In contrast to many other excellent methods of material science, NMR can operate at almost any pressure (as XPS and TEM cannot do), can penetrate the material depth (as, again, XPS and TEM and SEM and Raman etc cannot do), is not limited by disorder but can actually quantify it (as XRD cannot do), and can selectively detect components like Li ions (oblivious to most other methods). After having operated a Li-ion battery (the battery sort most used today), the Li in the carbon-based negative electrode is distributed over a large variety of poorly-characterised chemical environments that range from Li metal nanoparticles to the shadowy solid-electrolyte interphase (SEI). Many of those environments are sensitive to the slightest whiff of air or water and are distributed all across the 10^{-4} m depth of the electrode where the carbon component is often not crystalline. So…the stage is set for ^{7}Li NMR to study the fate of Li of Li-ion batteries.

Two recent results obtained mainly by ^{7}Li NMR are going to be presented. The first one [1] explores the most mundane property of NMR – namely, it is a rather good quantitative method. As it is going to be demonstrated, conventional spectrometers equipped with liquid-state probes are perfectly suitable for quantifying the amount of Li in various chemical environments in graphite-based electrodes and perform better and fast than most methods tried so far. This is particularly useful when one aims at the analysis of real aged batteries at hundreds of sample points. In real batteries, the reason for the appearance of ubiquitous macroscopic heterogeneities and connected capacity loss is quite unknown unknown.

The second study [2] concerns hard carbons as battery electrodes. Actually, we do not know what hard carbon is but use it because it has a higher capacity than graphite. Currently, there are several models, probably all amiss to a comparable extent. The hard carbon we studied was made into carbon fibers with the hope of being able to build “structural batteries”, that is batteries that may for example constitute the body of a car. Quadrupole-split NMR spectra and relaxation times recorded in a wide range of temperature and combined with Li self-diffusion data and, crucially, with data recorded at different orientations of the fibers relative to the magnetic field revealed not only a wide distribution of environments ranging from the rather ordered to the quite disordered with all those being contained in <10 nm domains but also a distinct mesoscopic order on the ~50 nm length scale with the ordered domains being cylindrically stacked.

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How NMR spectroscopy can help close the phosphate cycle

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The transition to a sustainable society requires a circular use of resources and thereby a need to understand the chemical reactions that govern the chemical transition from waste to resource. Phosphorus in the form of phosphate is a limited resource on the list of EU's critical resources, as it is an essential nutrient and phosphate fertilizers are needed to ensure sufficient food production. On the other hand, excess phosphate in freshwater systems leads to eutrophication and it is therefore removed at wastewater treatment plants to meet discharge limits.

The most efficient phosphate reuse requires that the recovery technology match the phosphate sources present. Selected steps spanning from a fundamental understanding of the properties of new materials to the mapping of phosphate resource at the wastewater treatment plant will be presented. Conventionally, phosphate speciation and the removal capacity of phosphate sorbents is based on analytical chemistry that provides limited insight into the chemical processes and composition on the atomic level, which may leave to incorrect conclusions. I will illustrate how NMR spectroscopy in combination with other advanced characterization techniques and analytical chemistry can provide the chemical knowledge needed to ultimately close the phosphate cycle illustrated by the two examples below..

1) The variations in the phosphate speciation of sewage sludge at several Danish wastewater treatment plants were mapped and linked to the phosphate removal strategies using a combination of sequential extraction combined with NMR spectroscopy, powder X-ray diffraction, electron-microscopy and elemental analyses, a recently developed protocol.[1] [2]

2) Sorbents are a promising technology for the selective capture of phosphate from wastewater and layered double hydroxides (LDH) related to the mineral hydrotalcite ($Mg_3Al(OH)_6(CO_3)2H_2O$) are some of the most promising materials. Here the phosphate sequestration pathway and efficacy of MgAl-LDH on the atomic level was established using multi-nuclear (^{1}H, ^{25}Mg, ^{27}Al and ^{31}P) solid state NMR spectroscopy revealing that the most efficient sorbent is not necessarily the best, as will be illustrated for different LDH sorbents.[3-6]

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Unravelling

Bcl-2 proteins´ functioning at mitochondrial membrane level

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Programmed cell death (apoptosis) is essential in life. In its mitochondrial apoptotic pathway opposing members of the B-cell lymphoma 2 (Bcl-2) protein family control the permeability of the mitochondrial outer membrane (MOM) and any release of apoptotic factors. Any imbalance can cause disorders including cancer, where upregulation of cell protecting (anti-apoptotic) Bcl-2 membrane protein itself plays a notorious role by blocking apoptotic proteins such as Bax. Here, we apply solid state/liquid state NMR spectroscopy and neutron reflectometry (NR) on supported lipid bilayers which mimic MOM environment and to unravel the molecular basis driving opposing proteins to interact with each other at the MOM; a mechanism which is not really understood yet due to lack of high-resolution structural insight. Based on our central hypothesis that Bcl-2 drives its cell-protecting function at a membrane-embedded location as revealed by NR (1), we focus i) to determine the structure of human Bcl-2 protein in its membrane setting by combining solution (2) and solid-state NMR; ii) use NR to study the kinetics and lipid/protein pore assemblied upon binding of Bax to mitochondrial membranes and its membrane destroying activities there; and iii) unravel the nature of direct interaction between Bcl-2 and Bax to neutralize each other. Knowledge generated here, will be indispensable in understanding the regulative function of the Bcl-2 family at mitochondrial membranes.

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The dynamic interaction of the N-Myc oncoprotein and the protein kinase Aurora A

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The intrinsically disordered N-Myc protein is a master regulator involved in numerous pathways important for cellular growth and function. However, when deregulated, this vital transcription factor is a key cancer driver[1]. Cellular studies have suggested that the protein kinase Aurora A disrupts N-Myc's path towards proteasomal degradation[2], increasing the risk of tumor development, and a fragment of N-Myc has through crystallography been shown to bind directly to Aurora A[3].

We have successfully produced the intrinsically disordered N-terminal region of the N-Myc protein, encompassing the conserved MB0 and MBI. By solution state NMR, we have for the first time characterized the N-Myc TAD region with respect to its dynamics and its transient structure. In addition, we have shown that the kinase domain of Aurora A binds directly and simultaneously to MB0 and MBI in a highly dynamic manner, displaying characteristics of a "fuzzy complex" while still forming a stable interaction as judged by SEC-MALS. Our ITC and NMR measurements jointly show that the interaction of N-Myc and Aurora A is independent of the region previously identified by crystallography. Furthermore, this interaction may be affected by a key cancer mutation in N-Myc, namely P44L.

Here, we will present our ongoing structural and dynamical analysis of the interaction between the intrinsically disordered N-Myc protein and the kinase domain of Aurora A, expanding our understanding of this oncogenic relationship.

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The right answer for the right reason: towards reliable predictions of paramagnetic NMR shifts

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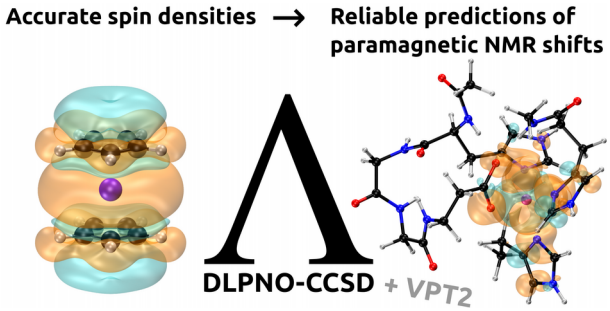

Electronic structure calculations are fundamentally important for the interpretation of nuclear magnetic resonance (NMR) spectra from paramagnetic systems that include organometallic and inorganic compounds, catalysts, or metal-binding sites in proteins. Prediction of induced paramagnetic NMR shifts requires knowledge of electron paramagnetic resonance (EPR) parameters: the electronic ***g*** tensor, zero-field splitting ***D*** tensor, and hyperfine ***A*** tensor. The isotropic part of ***A***, called the hyperfine coupling constant (HFCC), is one of the most troublesome properties for quantum chemistry calculations. Yet, even relatively small errors in calculations of HFCC tend to propagate into large errors in the predicted NMR shifts. The poor quality of ***A*** tensors that are currently calculated using density functional theory (DFT) constitutes a bottleneck in improving the reliability of interpretation of the NMR spectra from paramagnetic systems. Electron correlation effects in calculations of HFCCs with a hierarchy of *ab initio* methods were assessed in this work, and the applicability of different levels of DFT approximations and the coupled cluster singles and doubles (CCSD) method was tested. Severe deficiencies of DFT were revealed but the CCSD method was able to deliver good agreement with experimental data for all systems considered, however, at substantial computational costs. We proposed a more computationally tractable alternative, where the ***A*** was computed with the coupled cluster theory exploiting locality of electron correlation. This alternative is based on the domain-based local pair natural orbital coupled cluster singles and doubles (DLPNO-CCSD) method. In this way the robustness and reliability of the coupled cluster theory were incorporated into the modern formalism for the prediction of induced paramagnetic NMR shifts, and became applicable to systems of chemical interest. The effects of vibrational corrections to orbital shielding and hyperfine tensor were evaluated and discussed within the second-order vibrational perturbation theory (VPT2) framework.

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Identification of novel natural products possessing antibacterial and anticancer activities

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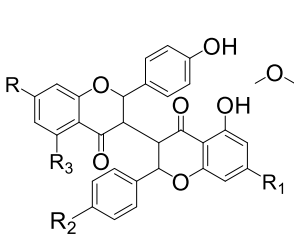
Abstract

We report the structure determination of 26 natural products including eight (**1-8**) new molecules, based on NMR spectroscopic and mass spectrometric analyses. In an endeavor to search for new bioactive natural products from Tanzanian medicinal plants, the stem bark extract of *O. holstii* and the root bark extract of *O. kirkii* were investigated. The structures and configurations of the molecules were established by extensive spectroscopic, ^{1}H and ^{13}C NMR and 2D (HSQC, HMBC, COSY, TOCSY and NOESY) experiments. The isolated metabolites were evaluated for antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) as well as for cytotoxicity against MCF-7 human breast cancer cells.



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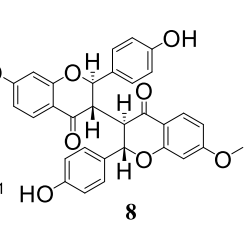

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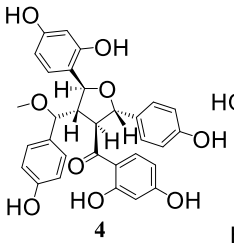

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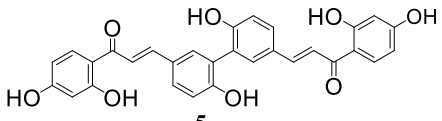

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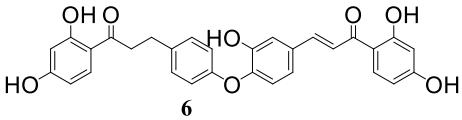

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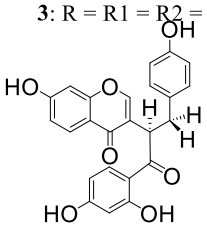

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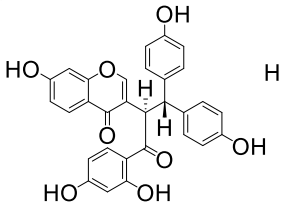

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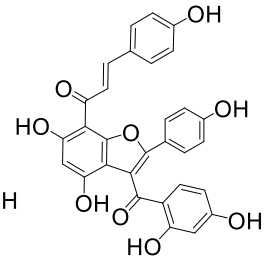

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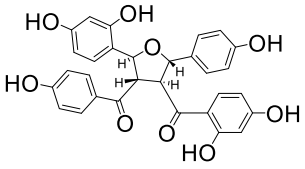

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Studying modified cellulose materials using DNP-NMR spectroscopy

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Cellulose is a polymer of natural origin and depending on the source the polymer material can vary widely in physicochemical properties. Therefore, consistent chemical modification of cellulose becomes a challenging task, and the final products tend to vary in their properties as a result. This put high demands on the methods used to analyse modified cellulosic materials. In this project we develop and apply, DNP-enhanced solid-state NMR spectroscopy for the study of modified cellulose materials. We focus mainly on cellulose ethers with industrial relevance and the use of correlation and spin-diffusion experiments to study molecular structure and spatial distribution of the etherifying substituents within the anhydroglucose units (AGUs) and also on a larger spatial scale within the cellulose particles. A fast and accurate analysis of these properties increases the possibility to correlate reaction parameters to product outcome in industrial production and leads to improved consistency and quality of the final products.

Cell-Free Protein Synthesis at the Swedish NMR Centre

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Cell-Free Protein Synthesis (CFPS) has been set up and used at the Swedish NMR Centre (SNC) for protein production since 2007. Now, being an important part of the Protein Production Sweden (PPS), a national-level research infrastructure aiming to produce superior quality protein samples, the CFPS platform at the Swedish NMR Centre offers a versatile toolbox for rapid and cost-effective *in vitro* protein synthesis.

The platform is capable of producing diverse types of protein samples (for e.g., soluble, membrane-bound, toxic) from a plasmid template using isolated translation machinery from bacterial (*E. coli*) extracts. NMR-optimized isotopic labelling for proteins through CFPS protocol with subsequent NMR data acquisition and analysis is also available. Current efforts are focused on the improvement of the current system as well as extending the CFPS applications through optimized protocols using yeast and mammalian extracts.

The methodology is available to local and external researchers upon request through PPS projects.

Characterization of a new rotenoid from the leaves of *Millettia oblata* ssp. *teitensis*

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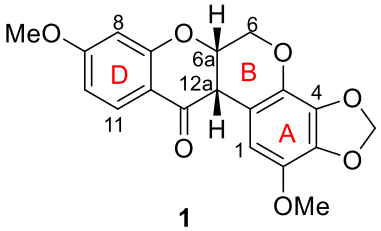
Abstract

A new rotenoid (**1**) was isolated from the leaves of *Millettia oblata* ssp. *teitensis* and characterized by NMR spectroscopic and mass spectrometric techniques. The skeleton of the structure was identified using 1D (^{1}H and ^{13}C,) and 2D (COSY, HSQC, HMBC, and NOESY) experiments. The unusual substitution in ring A of compound **1** was distinguished from an isomeric structure (**1a**) based on the ^{13}C NMR chemical shift of the methoxy carbon, the NOESY cross peak of the proton at position 1 with the nearby methoxy protons, and was confirmed by the HMBC correlation of H-1 with C-1a, C-2, C-3, C-4a, C-12a, and OMe with C-2. The small $^{3}J_{6a,12a}$ and the H-6a – H-12a NOESY cross peak enabled us to determine the relative configuration of compound **1** to *cis*.. The absolute configuration was established as 6a*S*, 12aS-**1** based on ECD spectroscopy.



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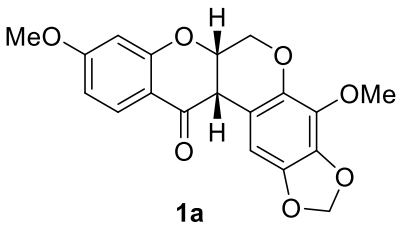

1a

Multi-scale structural investigations of protein materials

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In nature, proteins are the building blocks of many biomaterials with intriguing properties, e.g. silk and underwater adhesives. These materials have served as inspiration for manmade structures for long time but the challenge to produce synthetic materials with comparable properties from biobased resources remains. The key to achieve this is to gain control over the assembly of hierarchical structures from the protein building blocks.

We are exploring the formation of amyloid-like nanofibrils from agricultural resources and how these nanofibrils can be integrated in new materials. For this, multiscale structural analysis of the materials is needed, from the atomic details to the macroscopic appearances. We are combining several different methods (NMR, mass-spectrometry, X-ray scattering techniques, AFM, electron microscopy) and we have observed several examples of how the molecular and/or nanoscale structure of the fibrils can have substantial effects on the macroscopic material properties.

NMR provides a probe to investigate molecular features of the materials such as cross-linking mechanisms and conformational properties of the polypeptide chains. Although solid-state NMR is established as an important method to determine high-resolution structures of amyloid-like nanofibrils, the main focus has so far been on disease-related amyloids. We are exploring how the methodologies that were first developed for structural biology can be employed to provide information for the design of new materials.

The maintenance of mitochondrial DNA by TFAM as seen by solution NMR

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TFAM (mitochondrial transcription factor A) is a crucial DNA binding protein that plays an important role in regulating the mitochondrial DNA structure, similar to the nucleosome packaging by histones, as well as in the initiation of transcription of the human mitochondrial genome. The protein coats the mitochondrial genome forming them into dense spherical shapes named nucleoids by inducing sharp U-turns of the DNA. TFAM and mtDNA have been reported to create multiphase, gel-like structures by weak interactions inducing liquid-liquid phase separation (LLPS) that protects the mtDNA from oxidative damage within the mitochondrial matrix. TFAM consists of two high mobility groups (HMG) boxes, protein domains that both interact with DNA, and are mainly regulated by post transcriptional modifications on HMG1. TFAM function is highly regulated in mitochondria by site specific phosphorylation and targeted degradation by the homohexameric Lon protease. Nevertheless, the underlying functional mechanism of TFAM is still poorly understood and to unravel the underlying dynamic properties we used solution NMR spectroscopy as the main method in combination with different biophysical techniques such as BLI and SEC-MALS.

New Developments in Small Molecule NMR: From Fast-Pulsing 2D Experiments to Flexibility Determination Using Partially Aligned Samples

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Fast multidimensional NMR experiments are highly desired for a multitude of applications, including classical service-NMR. While ultrafast experiments, allowing to acquire a 2D in a single scan, suffer from several limitations, the simultaneous acquisition of several experiments and the application of fast-pulsing sequences that allow to heavily reduce the relaxation delay between scans are two viable approaches. The first part of the talk will focus on the latter experiments.

In the second part of the talk, NMR of partially aligned samples will be shortly introduced. Chemically synthesized gel-based alignment media made out of PEODA are introduced as well as a rheological device for influencing the alignment properties of PBLG as one of the most favored liquid crystalline phases for small molecules. Finally, the MDOC approach (molecular dynamics by orientational constraints) is explained and results for molecules with intermediate flexibility shown.

Metabolomics as a readout of the genotype

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Understanding the relationship between genotype and phenotype (i.e. properties or characteristics) is one of the central topics in modern biology. Fast developments in sequencing technologies enable researchers to obtain detailed genomic information.

We wanted to investigate the ability of the metabolome to provide a link between genotype and phenotype. Therefore, we performed NMR metabolomics on male flies from 170 different isogenic lines from the Drosophila melanogaster Genetic Reference Panel

Our results show that the metabolite profile is highly heritable, and we can identify genes associated with metabolome variation.

More interestingly, the metabolome gave better prediction accuracies than genomic information for four out of five phenotypes, even if the phenotypes and metabolites were measured on different flies in different labs and the only link between metabolome and phenotype is the genome.

This illustrates that NMR metabolomics has a large potential as predictor of organismal phenotypes and may be thought of as a decoder of the genome, a finding of great importance, e.g., in human medicine, evolutionary biology and animal and plant breeding.

The tale of loop-mutations: How a natural variant of the SARS-CoV-2 nucleocapsid protein enables detailed studies of the full-length protein by solution NMR spectroscopy

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Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is the third coronavirus that crossed from the animal kingdom to the humans in the twenty first century. Since its first detection in December 2019 in Wuhan China, the virus infected over 610 million people worldwide and caused more than 6.5 million deaths so far (https://coronavirus.jhu.edu). The high infectivity and severity of atypical pneumonia[1] together with other serious problems and consequences like disorientations and Parkinson's like symptoms[2,3] that last for the months after the infection, highlights the need for better understanding of viral life cycle and finding the therapeutics to lessen the severity of individual infection. Most of the therapeutical effort target viral entry, RNA synthesis[4] and protein processing but less emphasis has been on the other steps of the viral life cycle. One of those critical steps is the assembly of the viral genomic RNA and nucleocapsid protein (NCAP) into a ribonucleoprotein (RNP) complex. Therefore, a detailed understanding of the structure and the functional cycle of NCAP protein is uttermost importance since SARS-CoV-19 patients exhibit earlier and higher antibody response towards NCAP compared to the surface exposed spike protein. Here I present the full assignment and biophysical characterization of full-length wtNCAP as well as the so-called Italian/Austrian multinational variant of the viral nucleocapsid protein. In addition, I will also highlight for the first time the dynamic properties of the full-length protein as well as its interaction with nucleic acids and other binding partners.

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Isotopically labelled carbohydrates for NMR studies of protein-carbohydrate interactions

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Carbohydrates used in structural studies by NMR are rarely ^{13}C-labelled, preventing exploitation of the ^{13}C spectral dispersion in 3D or higher order NMR experiments, which would alleviate severe resonance overlap in their ^{1}H NMR spectra. This is in part associated with the lack of easily accessible isotope-labelled sugar molecules. In this presentation, I will describe our strategy for isotopic labelling, which is based on uniformly ^{13}C-labelled sugars and, in the case of aminosugars, also ^{15}N-labelling. I will discuss challenges with the production of isotopically labelled sugars, the lack of suitable NMR experiments for such compounds, and what information that can be collected from protein-carbohydrate interactions. With inspiration from protein NMR experiments, we are adapting NMR experiments for tailored analysis of isotopically labelled carbohydrates, including the measurement of *J*-couplings within the *N*-acetyl group of *N*-acetylglucosamine. [1] Our aim is to provide NMR tools for detailed characterization of glycan moieties in glycoproteins, carbohydrate-binding proteins, and carbohydrate-active enzymes. From protein-carbohydrate interactions, we can observe individual sugar hydroxyl protons in hydrogen bonds with the protein and thereby characterize the hydrogen-bonding network. [2-4]

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Through hardship to the multidimensional NMR and back again

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Multidimensional NMR experiments offer ultimate resolution and much information about interactions of atomic spins. Another major advantage of these experiments is that many spins in a complex molecule, e.g. a protein, are studied in parallel. However, there also high associated costs known as the curse of dimensionality, i.e. lengthy experiments, low sensitivity, and complicated data processing and analysis. We develop the non-uniform sampling (NUS) techniques and novel processing methods, including the compressed sensing and machine learning, to alleviate these problem.

There are many practical cases that allows to depart from the high dimensionality and the pertaining problems. We introduce a suite of 2D Focused SpectroscopY (FOSY) experiments as a fast, sensitive, and robust method to rapidly obtain information about selected spin systems of interest. By employing the long-overlooked concept of selective polarisation transfer (SPT), FOSY focusses onto one coupled nuclear spin system (e.g. a residue affected by PTM) at a time by selecting three to five known frequencies and, thus, provides the spectral dispersion equivalent to a 6D-7D experiment in only two dimensions and with an efficiency and versatility higher than achievable by traditional broadband experiments. We demonstrate the efficiency of FOSY by assigning, in just a few hours, two phosphorylation sites of proline-dependent glycogen synthase kinase 3 beta (GSK3β) in human Tau40, an IDP of 441 residues. The new approach will benefit NMR studies of protein hotspots, as sites involved in molecular interactions and conformational changes and resolve assignment bottlenecks.

Dmitry M Lesovoy, Panagiota S Georgoulia, Tammo Diercks, Irena Matečko-Burmann, Björn M Burmann, Eduard V Bocharov, Wolfgang Bermel, Vladislav Y Orekhov, *Unambiguous Tracking of Protein Phosphorylation by Fast High-Resolution FOSY NMR*, **(2021)** Angewandte Chemie, 133(44), 23732-23736

MAS DNP NMR for Pharmaceutical Formulation Characterization

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There is great interest in the development of improved drug-delivery systems that can provide targeted delivery of drug molecules, controlled release, or improve solubility of poorly soluble drugs. Since there are very few direct methods to probe the inside of these types of materials, DNP NMR is an attractive approach to look at these solid-state structures. Here we show how the use of relayed DNP NMR can investigate the molecular and macroscopic structure of pharmaceutical formulations.

Using ^{31}P and ^{19}F NMR to determine that the activity of a multi- selective trimeric adenylate kinase is dependent on large scale dynamics.

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The adenylate kinase from the archaea *Odinarchaeota* (OdinAK) are able to utilize all naturally occurring nucleotide tri-phosphates in order to phosphorylate AMP. This is in contrast to all other studied members of the adenylate kinase family. The reason for this broad selectivity is found in a long and flexible selectivity loop.

Different NMR techniques were employed to determine activity and dynamics in the approximately 68 kDa, thermostable trimeric enzyme. Using a combination of ^{31}P NMR, to determine the activity of the OdinAK, and ^{19}F NMR, to determine the conformational dynamics, we were able to show that the activity of OdinAK is restricted by the opening/closing dynamics of the ATP binding domain.

Automated metabolite quantitation by ^{1}H-NMR

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Nuclear magnetic resonance (NMR) spectroscopy is commonly used in metabolomics, for instance since ^{1}H-NMR analyses of complex mixtures allow metabolite quantitation. Although NMR-based metabolomics has many potential application areas, it is challenging to quantify metabolites. This specifically owes to signal interferences in data from complex mixtures. Interferences occur since complex mixtures contain many different compounds, each compound often has several signals, and some signals from different compounds positionally overlap. Unless accounted for, the interferences result in overestimated concentrations. Substantial efforts have been dedicated to develop methods that handle interferences. For instance, interferences from macromolecules can be limited by sample preparation[1-3], specialised experiments[3-5], or data processing[6-7]. Despite such efforts, some interferences between metabolite signals remain. Such interferences can be handled *in silico* using manual[8] or automated[9-13] approaches, that commonly adjust database signals from individual compounds, until their sum match the data. Automated procedures are more efficient than manual ones, but the computational burden tends to grow as the number of spectra and compounds to quantify increase[11]. Automated quantification algorithms (AQuAs) have been developed to account for metabolite interferences, allowing quantitation via an efficient data reduction strategy that avoids heavy computational burden[14-15]. Data generated for heparinised plasma has been used to demonstrate implementation of an AQuA[14] for efficient quantification of metabolites including sugars, amino- and organic acids. Algorithm evaluation has been based on quality indicators that reveal distributions of positions, interferences, and occurrences of selected signals. With data from plasma collected with EDTA as anti-coagulants, it has been further demonstrated how to improve the AQuA[15] to handle interferences also when signals display positional deviation.

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Structure and dynamics of the 600 kDa human mitochondrial protease Lon probed by solution NMR spectroscopy

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The Human Lon protein complex is an ATP-dependent serine protease involved in mitochondrial quality control residing in the mitochondrial matrix(1). Lon is a product of the nuclear gene *LONP1* and also plays a crucial role in the maintenance and repair of mitochondrial DNA as well as being an essential regulator of mitochondrial metabolism. Structurally, the hexameric human Lon protease consists of three distinct domains, an amino-terminal substrate recognition domain, a central AAA+ (ATPases associated with diverse cellular functions) protein unfoldase domain, as well as a carboxy-terminal serine-protease domain, features that only very recently could be revealed *via* single particle cryoEM(2).
Nevertheless, the underlying functional repertoire and especially the communication of the distinct parts of Lon remain largely elusive. To unravel these important properties, we have started to study the structural adaptations of Lon as well as its underlying dynamic properties and interacting machineries by using nuclear magnetic resonance (NMR) techniques. Due to the large size of the Lon protease with 600 kDa as the mature hexamer, extensive use of specific methyl labelling together with methyl TROSY spectroscopy was applied. The whole NMR study also exploited the modular assembly of the Lon protease by a divide-and-conquer methodology. I will show our initial insights into the structure and dynamics of the protease domain as well as a first glimpse of our studies or the amino-terminal substrate recognition domain.

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In-Cell Quantification of Drugs by MAS DNP NMR

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The determination of intracellular drug concentrations can provide a better understanding of drug function and efficacy. Ideally, this should be performed nondestructively, without modifying either the drug or the target. Moreover, sensitivity allowing the detection of low amounts of the molecule of interest, in many cases in the µM to nM range (pmol to fmol per million cells), is required. It is thus currently challenging to provide direct quantitative measurements of intracellular drug concentrations simultaneously satisfying these requirements. Here, we use an approach based on magic-angle spinning dynamic nuclear polarization (MAS DNP) which can fulfill these requirements. We apply a quantitative ^{15}N MAS DNP approach in combination with ^{15}N labeling to quantify the intracellular amount of [^{15}N]CHIR-98014, an activator of the Wnt signaling pathway, and we determine intracellular drug amounts in the range of tens to hundreds of picomoles per million cells. This is, to our knowledge, the first time that MAS DNP has been used to successfully estimate intracellular drug amounts.

A first peek at the Nordic Algae Cell Wall Structure

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Green microalgae are one of the most promising feedstocks for biofuels, food and high value compounds (e.g. pharmaceuticals) due to the diverse properties of their intracellular components. Compared to higher plants, microalgae produce high area yields, high oil content, they have low water consumption and the capacity to grow on arid lands. However, the harvesting and cell extraction steps are challenging bottlenecks for biotechnological applications, and these processes are directly linked with the microalgal cell wall. The cell wall needs to be broken down or enzymatically digested, a process that greatly adds to the energy exigencies of algal cultivation and exploitation. Understanding the cell wall composition is therefore very important, and yet, very little is currently known. Our research aims to explore the cell wall composition and morphology of four Nordic microalgal strains: Chlorella vulgaris (13- 1), Scenedesmus sp. (B2-2), Haematococcus pluvialis, and Coelastrella sp. (3-4) and their changes in relation to logarithmic and stationary growth phases.

We have used transmission electron microscopy imaging to visualize the cell walls and to observe structural elements for the different growth stages, Cryo-XPS to quantify lipid, protein and polysaccharide content of the outer surface of the cell wall, FT IR spectroscopy to highlight changes between growth phases [1], and in this presented study, inspired by Arnold et al.[2], various solid state NMR methods to obtain a molecular detailed and dynamically filtered view of the algal cell wall composition at different growth stages.

The algae was grown for 5 and 10 days in a media containing 13C $NaHCO_3$ as the carbon source. Ultra- centrifuged whole cell wet pellets where transferred into 2.5 mm MAS rotors and a range of 13C detect solid state NMR experiments (1D CP, DP NOE, RINEPT and 2D CP DARR and RINEPT TOBSY) were performed using a Bruker AVIII 500MHz spectrometer. The spectral analysis and interpretation is ongoing and some initial conclusions will be presented.

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In-situ characterization of the bacterial outer membrane protein A

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Outer membranes of Gram-negative bacteria contain transmembrane proteins in a distinguished membrane environment of highly asymmetric architecture. Whereas the inner leaflet of outer membranes is composed of regular phospholipids, the outer leaflet consists almost exclusively of lipopolysaccharides (LPS). To date, no methods exist to experimentally mimic the complex native lipid environment of bacterial outer membranes. Studies characterizing the structure and function of outer membrane proteins rely on membrane-mimetic systems or artificially reconstituted bilayers. However, a growing number of studies indicate that the membrane composition directly impacts the structural and functional states of integral membrane proteins.

To address this crucial issue, we recently developed a method to manipulate the protein content of bacterial outer membrane vesicles (OMVs). These vesicles are prepared to contain selected outer membrane proteins at high density and circumvent the limitations of established methods. Using engineered OMVs, we here characterize the prototypic outer membrane protein A (OmpA) from *Escherichia coli* for the first time within its native asymmetric membrane environment at the atomic level using proton detected ultra-fast solid-state NMR spectroscopy. Our methodology allows extensive assignment and structure determination of the transmembrane part of OmpA and site-specific comparison with OmpA reconstituted in lipid bilayers. An increase in detectable resonances by > 50% as well as increased backbone order parameters indicate that OmpA has a different, more stable and extended fold within the native membrane environment compared to existing structures in membrane-mimetic systems. Our work thereby provides a blueprint for *in situ* structural biology of membrane proteins in the future.

Substrate binding and protonation states at the active-site of a nickel superoxide dismutase-derived metallopeptide: implications for the mechanism of superoxide degradation

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Small, catalytically active metallopeptides, which were derived from the nickel superoxide dismutase active site were employed to study the mechanism of superoxide degradation[1] especially focusing on the protonation states of the Ni(II) donor atoms, substrate binding, proton source and the role of the N-terminal proton(s).[2] Therefore, we studied the Ni(II)-metallopeptides at various pH and temperature using UV-Vis and solution and solid-state NMR. These studies indicate a strong reduction of the pK_a of the Ni(II)-ligating donor atoms resulting in a fully deprotonated Ni(II)-active site environment.[2] Further, no titratable proton could be observed within a pH ranging from 6.5 to 10.5.[2] This rules out a recently discussed adiabatic proton tunneling-like hydrogen atom transfer process for the metallopeptides, not found in the native enzyme.[3] Furthermore, variable temperature ^{1}H NMR measurements uncovered an extended hydrogen bond network within the Ni(II) active site of the metallopeptide similar to the enzyme. With respect to the deprotonated Ni(II)-active site, the residual N-terminal proton, which is a prerequisite for catalytic activity, cannot act as proton source. Most likely, it stabilizes the Ni(II)-coordinated substrate in an end-on fashion thus allowing for an inner-sphere electron transfer.[4] Unlike the enzyme, the catalytic rate constant of superoxide degradation by the metallopeptides was determined to be strongly pH dependent suggesting bulk water to be directly involved in proton donation, which in turn strongly suggests the N-terminal histidine to be the respective proton donor in the enzyme.[5] Lastly, the position of the substrate within the active site environment was established through REDOR-NMR employing a set of isotope labeled NiSOD mimics including cyanide bound to the metal center as a substrate analog.[6]

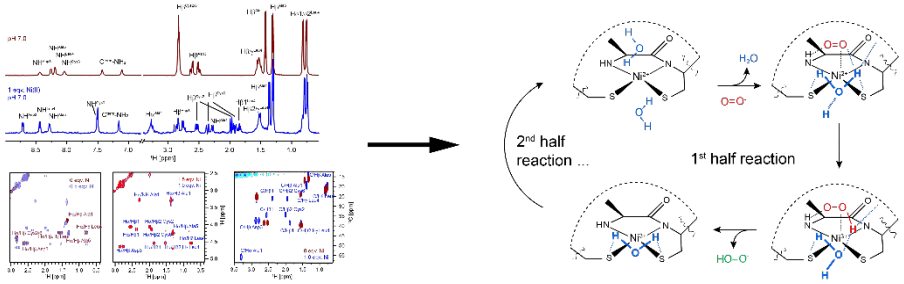

Figure 1: Ni(II) active site protonation and mechanism of O_2^- degradation revealed by NMR

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Paramagnetic ligand tagging to identy ligand-protein binding sites

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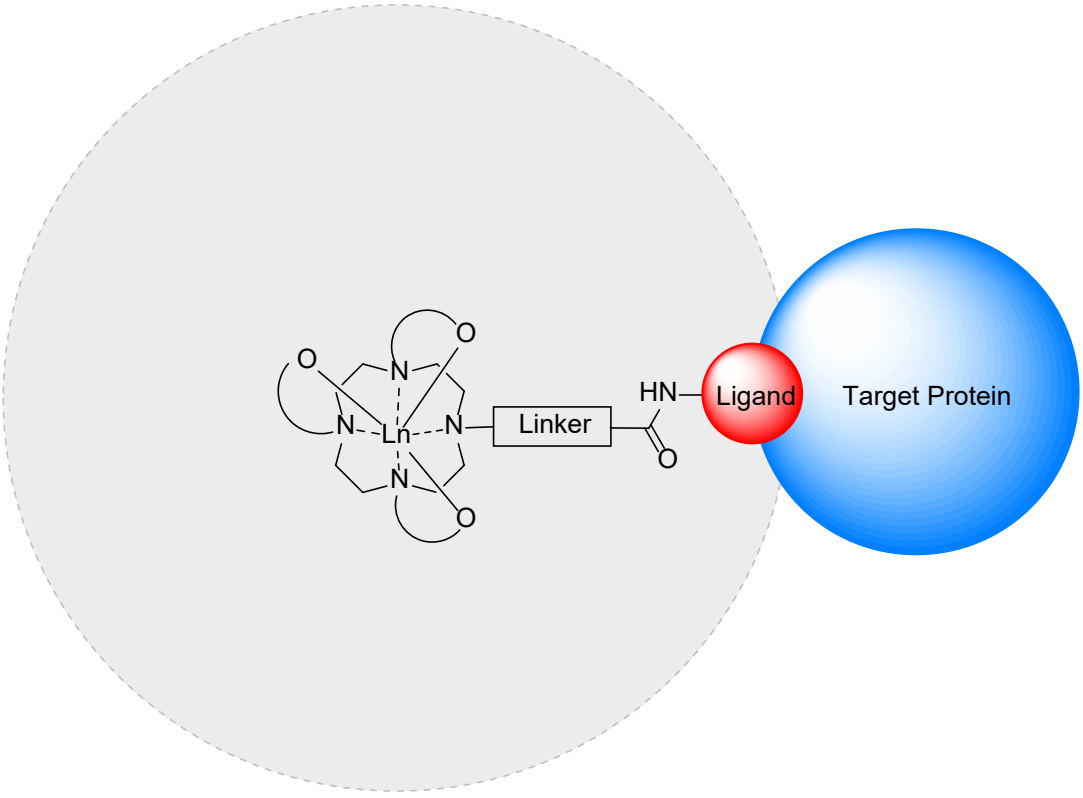
Ligand-protein interactions are pivotal in living organisms. Knowing the binding sites of small-molecule ligands, especially low affinity ones, is an important step for modern drug discovery as it enables the development of high affinity lead compounds. Due to the lack of robust characterization approaches, the determination of weak binding is still cumbersome. We have reported a paramagnetic ligand tagging method by taking advantage of pseudocontact shifts of protein NMR, which requires the ligand to be linked to a paramagnetic lanthanide complex.[1] In this project we have improved the structure of our original paramagnetic tag to increase the accuracy of the NMR-based structure determination as compared to our previous approach.



1. U. Brath, S. I. Swamy, A. X. Veiga, C. C. Tung, F. Van Petegem, M. Erdelyi, *J Am Chem Soc* **2015**, *137*, 11391-11398.